Exploring the Structure of a Hydrogen Cyanide Polymer by Electron Spin Resonance and Scanning Force Microscopy

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Summary: Aqueous solutions of potassium cyanide and ammonium hydroxide are known to yield a heterogeneous cyanide polymer, containing paramagnetic sites and biologically significant substructures including polypeptides. Here, such solutions were used to prepare various samples of polymer for study by X-band and W-band electron spin resonance (ESR), scanning electron microscopy (SEM), and scanning force microscopy (SFM). Elemental composition of a typical sample of the polymer was C-35.2%, N-38.47%, O-14.51%, and H- 4.13%, exposing the polymer to 6M HCl hydrolyzed portions of the polymer and released glycine and traces of other amino acids. The X-band ESR spectra consist of a single slightly asymmetric line centered at g = 2.003; spin concentration measurements made at X-band using a nitroxide radical standard yield approximate radical concentrations of 10^{18} spins/gm. W-band ESR indicates the presence of a single rhombic paramagnetic site with $g_x = 2.0025$, $g_y = 2.0030$, and $g_z = 2.0048$ and the possibility of small ^{14}N hyperfine splittings. The ESR spin echo studies yield a longitudinal relaxation time, T_1, of 75 µS and a short-phase memory relaxation time, T_m, of about 300 nS. Scanning electron microscopy studies of the polymer show that it is made of ellipsoidal particles about one micron in size. The particles tend to clump together when suspended in aqueous solution. The particles disperse and dissolve in dimethyl sulfoxide (DMSO); when these solutions dry on microscope slides, optical microscopy shows a branched island morphology for the polymer. This morphology is reminiscent of snowflakes and is identified as dendritic. Phase contrast SFM of the dendritic arms show a striking segregation and ordering of various components of the polymer. Paramagnetic sites are conserved in the series of steps leading to dendritic structures.

Key words: cyanide, polymer, resonance, scanning force microscopy

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Introduction

Radio astronomy shows that H_2O, HCN, and NH_3 are among the most abundant molecules in the universe. Thus, the chemistry of these species is of interest to the scientific community investigating planetary atmospheres, chemical evolution, and prebiotic chemistry (Delsemme 1998). Particularly important is the discovery by Oro *et al*. that adenine can be formed in aqueous solutions of HCN and ammonia maintained at temperatures below 373 K (Oro 1960, 1961; Oro and Kimball 1962). The reaction conditions which produce adenine, when maintained for a period of days, also produce cyanide polymer as a reaction product (Volker 1960). Moreover, what appear to be similar materials can be produced by an electrical discharge in gaseous HCN, or by adding base to liquid HCN (Mathews 1992, Minard *et al*. 1998). In general, the polymeric materials formed in these reactions seem to be heterogeneous in color, structure, and chemical properties depending on the conditions under which the polymer is formed (Ferris *et al*. 1981, Liebman *et al*. 1995, Minard *et al*. 1998). Minard *et al*. (1998) have used a variety of experimental techniques to probe the structure of HCN polymers and shown that these polymers can yield several nitrogen heterocycles, including adenine derivatives closely related to biologically important molecules. Liebman *et al*. (1999) reported that HCN polymers, formed under a variety of conditions, contain a substantial concentration of free radical sites which are stable in the presence of oxygen. This group reported that polymeric material stored for over 8 years at room temperature gave a strong electron spin resonance (ESR) signal.

The first objective of the work reported here is to use the results of both high-field and pulse ESR experiments to

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learn more about the structure of the free radical sites in hydrogen cyanide (HCN) polymers. The polymeric material studied in our laboratory, formed under conditions similar to those described by Oro, could be connected to the "lagoon" model for prebiotic synthesis (Oro 1960, 1961). An understanding of the structural features of the free radical sites which form in cyanide polymers is of interest from at least three perspectives. First, the very existence of radicals may indicate that a free radial process contributes to the formation of at least portions of the heterogeneous polymer. Second, the number and nature of free radical sites present in a cyanide polymer will strongly influence nuclear spin relaxation times and chemical shifts of certain nuclei in molecules of that polymer. Such influences will be reflected in the solid state nuclear magnetic resonance (NMR) of the polymer. Third, under the proper conditions the existing free radical sites may be capable of undergoing reactions with new reagents coming in contact with the polymer. Electron spin resonance results might provide insight into this polymer formation process.

The second objective of our research is to use scanning electron microscopy (SEM), optical microscopy, and scanning force microscopy (SFM) to image the structures formed by the polymer as synthesized, and by the polymer after solutions of the polymer are allowed to dry on glass slides. Such images provide information on the role of heterogeneity in determining polymer properties and on the ability of the polymer to form organized structures.

Materials and Methods

Potassium cyanide, KCN, (ACS Grade) and $K^{13}CN$ (99%) were obtained from Sigma-Aldrich Chemical Company, Inc. (St. Louis, Mo., USA); ammonium chloride, NH_4Cl, and dimethyl sulfoxide (DMSO) were obtained from Baker Chemicals (Phillipsburg, N.J., USA), and were ACS Grade. The water used throughout the experimental process was obtained from a Barnsted Nanopure water system (Barnsted Nanopure, Boston, Mass., USA) and had conductivity below $18.5.M - Ohm\ cm^{-1}$. The D_2O used in our experiments was obtained from the Isotec Division of Sigma Aldrich. The synthesis was carried out by preparing 100 ml of an aqueous solution, 1.0 to 1.5 M in both KCN and NH_4Cl, in a 250 ml round bottom flask. The flask was attached to a condenser and the solution was heated to 343 K for approximately 80 h. It is important to note that the reaction mixture was not stirred. The reaction was exposed to oxygen under ambient conditions (590–600 torr average atmospheric pressure). The polymer was isolated by centrifuging the reaction mixture at 4000 rpm for 10 min then pouring off the liquid phase and resuspending the polymer in water. This process was repeated a total of three times, with the polymer being isolated after the final centrifugation. Removal of salt from the polymer was verified by testing the liquid above the centrifugate with an $AgNO_3$ solution. Essentially, the same material was obtained from polymer preparations in which salt was removed from the polymer by dialysis. The polymer was dried in a vacuum desiccator. The material formed in this manner was virtually insoluble in water, but dispersed and dissolved readily in DMSO to produce a black solution which did not change in appearance when left standing for a period of weeks. Elemental analysis of a typical sample of the polymer was carried out by Galbraith Laboratories, Inc. (Knoxville, Tenn., USA).

The X-band ESR experiments were carried out on a Bruker ESP-300E spectrometer (Bruker Instruments, Billerica, Mass., USA). Spin concentration measurements were made using a solution of TEMPOL (Aldrich Chemical) in benzene. The purity of the TEMPOL was determined using the optical absorption at 429 nm (Kooser *et al*. 1992). The W-band and X-band pulse as well as W-band cw experiments were performed with a Bruker E 680 X/W spectrometer. T_1 was measured by saturating the spin system with a comb of 12 hard (12 ns) pulses and monitoring the recovery with a Hahn echo. The phase memory relaxation time, T_M, was measured by observing the echo decay using soft (80 ns) pulses to minimize instantaneous diffusion effects.

All SFM images were acquired in intermittent contact mode using a Park Scientific instruments auto-probe (now Veeco Metrology Group, Santa Barbara, Calif., USA). The substrates upon which the cyanide polymer samples were deposited were glass microscope coverslips cleaned with acetone. For SFM experiments, solutions of small amounts of cyanide polymer in DMSO were deposited on the substrate and allowed to dry under reduced pressure in a vacuum desiccator. Samples prepared in this manner were examined under the optical microscope of the auto-probe; photographs of the microscope images, as displayed on a TV monitor, were obtained using a 35 mm Olympus OM-1 (Olympus America, Melville, N.Y., USA). The SEM images were obtained from aqueous suspensions of polymer which were dried on a microscope slide. A LEO 435VP SEM operating at 15 kV was used to acquire the images (LEO Electron Microscopy Ltd., Cambridge, England).

Experimental Results

Discoloration of the reaction solution and formation of small amounts of polymer occurred within 2 h of mixing the reagents. While not explicitly stated in the literature, it seems reasonable to assume that the free radical sites observed by Liebman *et al*. (1999) arise from air oxidation of nitrogen in an easily oxidized substructure of the polymeric material. In a test to determine the role of oxygen in the reaction, a reaction mixture like that used to form the polymer was degassed on a vacuum line and allowed to sit for a period of several days. No sign of discoloration or polymer formation occurred until the system was exposed to oxygen under ambient conditions. Further experiments concerning the role of oxygen in the synthesis of polymer and the generation of paramagnetic sites are planned for the future.

The elemental analysis of a typical sample yielded: C $36.5 \pm 0.9\%$; H $4.2 \pm 0.1\%$; N $39.2 \pm 0.7\%$; O $15.7 \pm 1.6\%$. The errors reported are those associated with the elemental analysis. Approximately 10% of the polymer (by mass) could be hydrolyzed by digesting polymeric material in 6 molar HCL for 24 h at 383 K. Analysis of the hydrolyzed material showed that about 70% of it consisted of glycine; other amino acids detected in trace amounts included L,D-Ala; L,D-Glx; and L,D-Ser. The fact that a racemic mixture of these trace amino acids appeared in the analysis indicates that they are not present due to contamination of the polymer by biologic material. Figure 1 shows an SEM image of the material produced by the synthesis; an analysis of this image shows the polymer to be made up of ellipsoidal particles about one micron in size.

All ESR data presented were representative of numerous spectra obtained on our samples. The X-band ESR spectrum of the polymeric material consisted of a single slightly asymmetric line centered at g = 2.003; a spectrum of this type is shown in Figure 2. Formation of the polymer using K^{13}CN produced polymer samples which yielded ESR spectra having a line width of 14.5 Gauss, while preparation of a polymer sample from ND$_4$Cl solutions in D$_2$O produced material which yielded single-line ESR spectra having a line width of 6.0 Gauss. These experiments lead to the simple conclusions that the unpaired electron is delocalized over a structure containing one or more ^{13}C nuclei and that unresolved proton splittings contribute only slightly to the overall line width. Spin concentration measurements on various samples produced values between 2.5 E17 and 8 E18 spins/gm. In general, high reactant concentrations, long reaction times, and higher reaction temperatures lead to an increase in spin concentration. The sample with the highest spin concentration was prepared from 1.5 M KCN and NH$_4$Cl and allowed to react at 340 K for 80 h.

It was not possible to observe an ESR spectrum from the DMSO solutions of the polymer; however, the solid material remaining after evaporation of the DMSO gave a spectrum identical to that of the initial polymer. This observation shows that at least the portion of the polymer containing unpaired spins is not degraded in solution and suggests that the SFM images presented below are representative polymer material which has not undergone significant chemical change. The failure to observe a signal from the solution in the liquid phase arises because it is only possible to tune the ESR spectrometer with a small DMSO/polymer sample, and the concentration of the polymer in the DMSO is relatively low ($\sim 10^{-3}$ g /ml DMSO). Motional broadening arising from the large size of the polymer fragments and the high viscosity of the solvent may also play a role.

The W-band ESR spectrum of a sample of the cyanide polymer is given in Figure 3. An analysis of this spectrum shows that the dominant paramagnetic site is rhombic with $g_x = 2.0025$, $g_y = 2.0030$, and $g_z = 2.0048$; simulations of the experimental spectrum assuming splitting from a single ^{14}N nucleus wit h Az ~ 6 Gauss, Ax = Ay ~ 4 Gauss, a
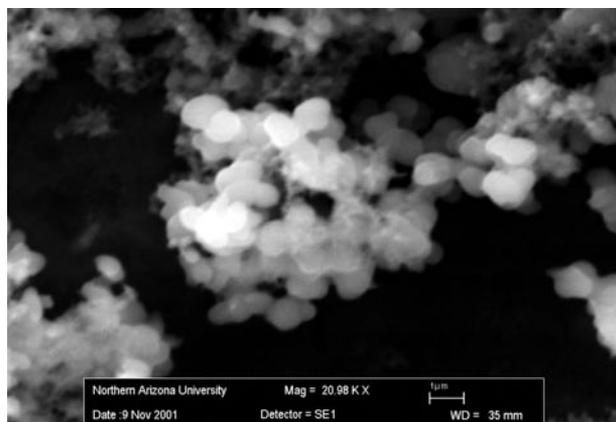

FIG. 1 Scanning electron microscopy image taken at 21.98k x of polymer material suspended in water and dried on a glass microscope slide.

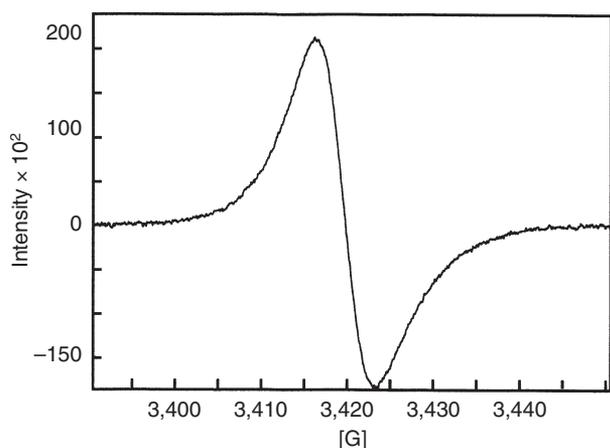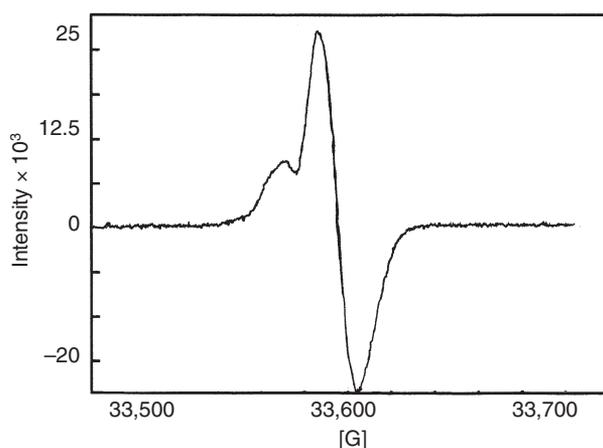FIG. 2 X-band ESR (ν = 9.605 GHz, M.A. = 2 × 10^{-2} mT) of cyanide polymer at 24°C and under ambient atmosphere.

FIG. 3 W-band ESR(ν = 94.18 GHz) of cyanide polymer at 24°C and under ambient atmosphere.

linewidth of 10 Gauss, and a lineshape half Lorentzian and half Gaussian gave a reasonable fit to the experimental spectrum. Two batches of the polymer, prepared several months apart in the manner described in the experimental section, gave spectra essentially identical to those shown in Figure 3. Pulse ESR studies yield a T_1 of 75 μs and a T_M of 300 ns. The T_1 value is not unusual for an organic radical; however the T_M value is unusually short. Further analysis may reveal whether the T_M values can provide useful information on the polymer structure or dynamics. The pulse studies showed no evidence for more than one type of paramagnetic site in the samples studied.
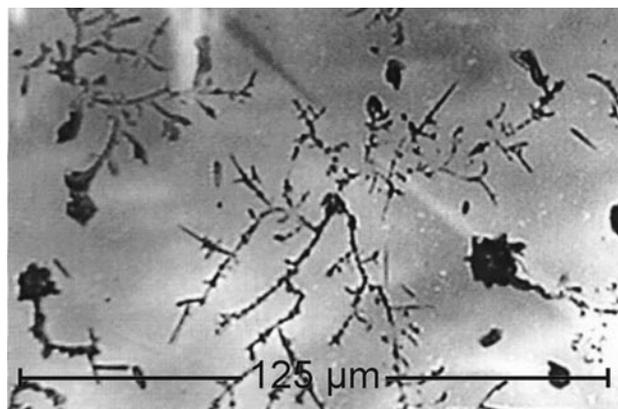

FIG. 4 Microscopic image of dendritic structures formed by cyanide polymer deposited on a glass coverslip from a dimethyl sulfoxide solution. Note the two central structures which show arms radiating from a hexagonal core.
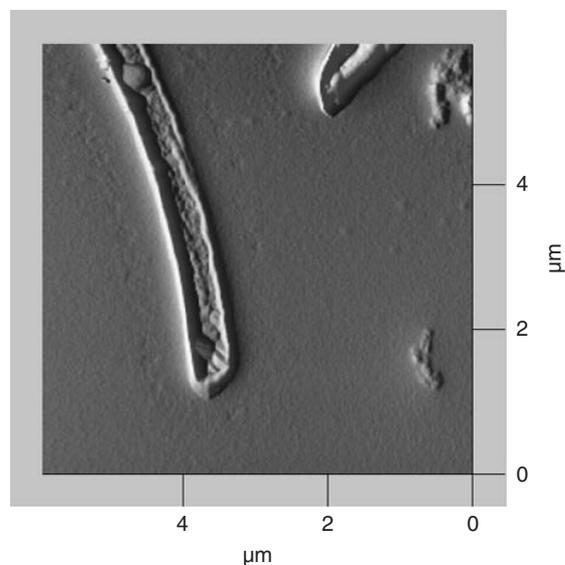

FIG. 5 Intermittent contact mode scanning force microscopy image (5 × 5 μm) of a tubule of cyanide polymer deposited on a glass cover slip from a dimethyl sulfoxide solution. The lower left-hand portion of the figure shows a tubule with the top section collapsed, exposing what appears to be a hollow core. In the upper right-hand portion of the figure is a tubule section with what appears to be an uncollapsed section.

Figure 4 shows an optical microscopic image of the results of allowing a solution formed by allowing a DMSO solution of the polymer to dry on a glass coverslip. This figure shows clear evidence of branched structures about 20 μm in size, with 2 μm thick arms which appear to be growing off hexagonal cores.

Figure 5 shows an SFM image of a small arm approximately 0.5 μm in width and about 4 μm in length coming off a dendritic structure like those in Figure 4. The major feature in the image shows what appears to be a collapsed tubule. In the upper right hand side of the figure is a structure which suggests a fully enclosed tubule. The images do not provide information on whether the enclosed structures can include liquid. Also unknown is the structure of the tubule walls and the extent to which they are permeable to various species in solution.

The SFM images in Figures 6–9 provide information on the structure of an uncollapsed tubule segment. Here we see in Figures 6 and 8 (topographical SFM mode) that the tubule has a smooth surface morphology. Images taken in phase contrast mode (Figs. 7 and 9) show striking chemical and structural inhomogeneities as one moves across the tubule. Phase contrast imaging allows for the differentiation of material properties on a sample surface in addition to simple topographical information. In these images, the dendrites are seen to be composed of smaller subunits or substructures. It appears that some level of self assembly is occurring as the dendritic arms form.

Discussion & Conclusions

It has been demonstrated that under "lagoon"-like conditions, KCN and NH_4Cl react to form ellipsoidal structures on the order of 1 μm in size. Our ESR data on these structures provide us with at least partial understanding of the role of free radical sites in their formation. First, these structures do contain paramagnetic (free radical) sites, which seem to be uniform in terms of their magnetic parameters. Second, we have determined that the paramagnetic (free radical) sites occur in a ratio of about 1 paramagnetic site/20,000 HCN monomer units. Assuming the distribution of sites is uniform in the polymer, and that the unpaired spin density is not distributed over a large molecular framework, one expects that these sites are essentially noninteracting. Our ESR results strongly suggest that such conditions do indeed apply in the polymer. Third, the location of these sites in the large-scale structure of the polymer remains unknown, as is their precise role in the polymerization process or in the subsequent reactivity of the polymer. In aqueous systems, these structures form a mass which is probably held together through hydrogen bonds involving water; specific solvents such as DMSO are capable of dispersing and dissolving the polymer. Allowing these solutions to dry on glass slides produces interesting dendritic structures.

Our SFM images allow us to study the macroscopic structure as well as the substructure of the HCN polymer.

The branched island morphology of the cyanide polymer deposit grows from hexagonal prism cores that form on the substrate. This growth mode is reminiscent of formation of snowflakes, ice crystals, and dendrites of other substances (Ben-Jacob and Levine 2001, Bentley and Humphreys 1962). Dendritic structures like those in Figure 4 generally arise from nonequilibrium situations and depend on the diffusional properties of the structural units in solution, and their ability to be incorporated into the dendrite at the growth front. In the present case these structural units may be segments of polymer dissolved in solution. Formation of dendritic morphology can be described mathematically using Mullins-Sekerka instabilities (Mullins and Sekerka 1964).

In the present case dendritic clusters (DC) grow in a process primarily dominated by diffusion of constituent units (dissolved polymer segments) to areas of rapid growth. In the initial stage individual clusters grew inde-
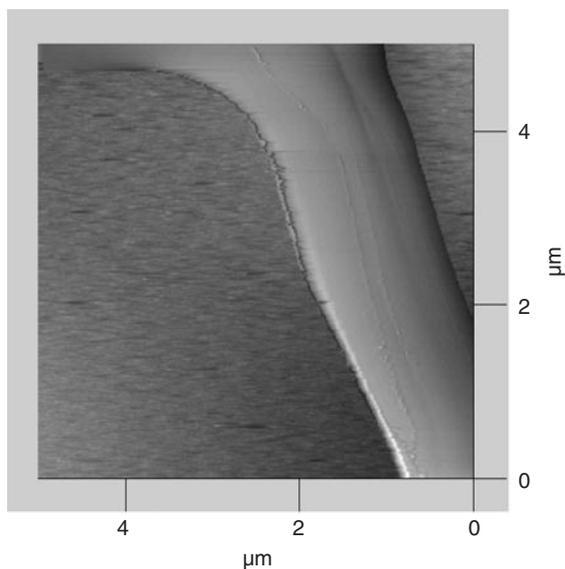

FIG. 6   Intermittent contact mode scanning force microscopy image (5 × 5 μm) of a single dendritic arm. Standard topographical image shown no substructure.
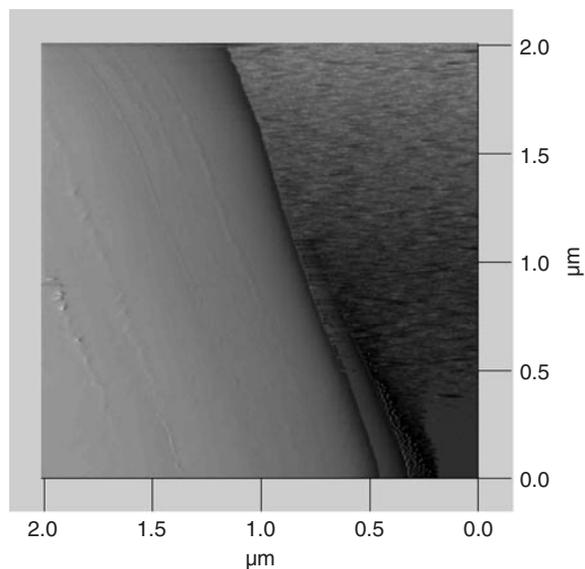

FIG. 8   Scanning force microscopy topographical image (2 × 2 μm) of cyanide polymer sample deposited on glass from a dimethyl sulfoxide solution. This is a section of the tubule shown in Figure 6. Note that the smooth topology evident in Figure 6 is also present in this image.
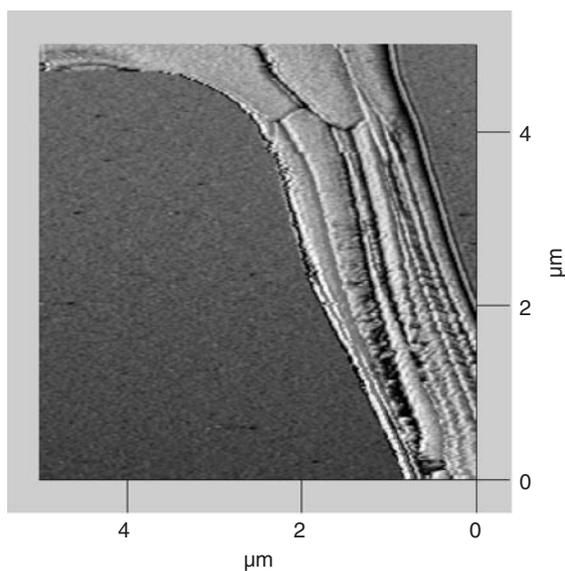

FIG. 7   Phase contrast scanning force microscopy image of the same region in Figure 6. Here, the dendrite is seen to contain a rich, repeating substructure.
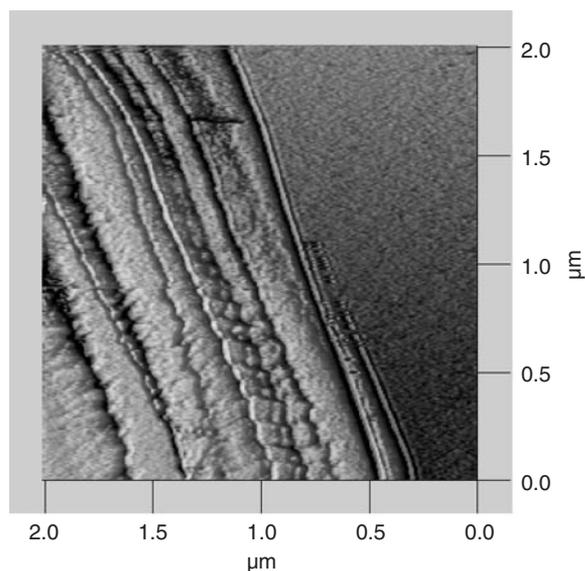

FIG. 9   A phase contrast scanning force microscopy image of the same tubule section shown in Figure 8. Note the organization of strands and the apparent segregation of various materials in the tubule.

pendently from each other. After reaching the size comparable with the average distance between the cores, the well-developed clusters begin interacting and may merge to form a multicore cluster. Dendritic cluster growth is essentially nonlocal in nature, that is, the growth rate in a given point depends on the entire geometry of the cluster, not only on the local morphology. The nonlocality of DC is a result of the shadowing generated by the branches of the cluster, which is easily recognizable in Figure 4, for example, absence of the left branch of an otherwise six-fold cluster in the upper-right corner. Of importance is the fact that the hexagonal symmetry of growing DC is a reflection of the symmetry exhibited by the polymer units as they form crystalline material.

There are several aspects of the cyanide polymer structure and chemistry which could be important in future origin of life studies. Zubay (2000) has suggested that "Selection pressure favors any chemical system that can process matter more rapidly and make more of its kind." The rapid growth of dendritic systems, as opposed to globular crystals, occurs because of kinetic processes. The most important process is diffusion of reactants to growth fronts which penetrate the reaction medium. Breaking of the arms of DC at bottleneck points, that is, "fragmentation" of DC, and transfer of the shredded pieces by hydrodynamic flows to new sites with the potential for new cluster growth, represents in a sense a type of reproductive process. The polymer systems studied here have structures with large surface areas; the tubules may include solutions and enable the transportation of dissolved species from one region of the structure to another. The SFM phase contrast images also suggest aspects of self assembly in the formation of the dendritic arms. It is possible that the varied structural and chemical features of the dendrites produce an array of microenvironments for subsequent reactions, and that the structures and chemistry of the polymer can undergo evolutionary processes if left for extended periods. At present, it is unknown to what extent the tubule walls are permeable to solvent, ions or dissolved organic species. It is also unknown to what extent morphology and reactivity depend on the presence of trace metals and organic reactants. Preliminary experiments in our laboratory have shown that the polymer strongly complexes a variety of transition metal ions. The images presented provide further evidence that understanding the free radical chemistry and structural features of cyanide polymers could be useful in further development of models for prebiotic chemistry.

Acknowledgments

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